Stem Cell Research 29 (2018) 143–147

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

Generation and characterization of two human iPSC lines from patients with methylmalonic acidemia *cblB* type

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ARTICLE INFO

Article history:
Received 28 February 2018
Received in revised form 20 March 2018
Accepted 29 March 2018
Available online 5 April 2018

ABSTRACT

Two human induced pluripotent stem cell (iPSC) lines were generated from fibroblasts of two siblings with methylmalonic acidemia *cblB* type carrying mutations in the *MMAB* gene: c.287T➔C (p.Ile96Thr) and a splicing loss-of-function variant c.584G➔A affecting the last nucleotide of exon 7 in *MMAB* (p.Ser174Cysfs*23). Reprogramming factors *OCT3/4*, *SOX2*, *KLF4* and *c-MYC* were delivered using a non-integrative method based on the Sendai virus. Once established, iPSCs have shown full pluripotency, differentiation capacity and genetic stability.

Resource table

Unique stem cell lines identifier	UAMi002-A UAMi003-A
Alternative names of stem cell lines	MMAB35-FiPS4F4 (UAMi002-A) MMAB44-FiPS4F11 (UAMi003-A)
Institution	Centro de Biología Molecular Severo Ochoa UAM-CSIC, Universidad Autónoma de Madrid, CIBERER, IDIPaz, Madrid, Spain.
Contact information of distributor	Belén Pérez, bperez@cbm.csic.es
Type of cell lines	iPSC
Origin	Human
Cell Source	Fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus
Multiline rationale	Derived from two siblings
Gene modification	No
Type of modification	Non applicable
Associated disease	Methylmalonic acidemia *cblB* type
Gene/locus	*MMAB*/12q24.11
Method of modification	Non applicable
Name of transgene or resistance	Non applicable
Inducible/constitutive system	Non applicable
Date archived/stock date	October 2017
Cell line repository/bank	Spanish National Bank of Cell Lines http://www.isciii.es/ISCIII/es/contenidos/fd-el-instituto/fd-organizacion/fd-estructura-directiva/fd-subdireccion-general-investigacion-terapia-celular-medicina-regenerativa/fd-centros-unidades/fd-banco-nacional-lineas-celulares/fd-lineas-celulares-disponibles/lineas-de-celulas-iPS.shtml
Ethical approval	Patient informed consent obtained. Ethics Review Board-competent authority approval obtained (CEI 48-919)

Resource utility

The iPSC lines were generated to investigate the pathophysiology of methylmalonic acidemia *cblB* type disease. In addition the effects of selected therapeutic compounds will be evaluated on disease-specific iPSC derived hepatocytes and neurons.

Resource details

Methylmalonic acidemia *cblB* type (MMA *cblB* type) is an inherited metabolic disease caused by mutations in the *MMAB* gene encoding ATP:cob(I)alamin adenosyltransferase that catalyses the synthesis of

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https://doi.org/10.1016/j.scr.2018.03.021

adenosylcobalamin (AdoCbl) from cobalamin using ATP (Froese and Gravel, 2010). Fibroblasts from two siblings carrying the mutations c.287T➔C (p.Ile96Thr) and c.584G➔A (p.Ser174Cysfs*23) (Table 1) were reprogrammed using the CytoTune™ iPS Reprogramming kit delivering the four human reprogramming factors *OCT3/4*, *SOX2*, *c-MYC* and *KLF4* (Takahashi et al., 2007). The iPSC lines MMAB35-FiPS4F4 (UAMi002-A) and MMAB44-FiPS4F11 (UAMi003-A) displayed a typical round shape ESC-like morphology and growth behaviour (Fig. 1A, Table 2) and the colonies stained positive for alkaline phosphatase activity (Fig. 1B). The clearance of the vectors and the exogenous reprogramming factor genes was observed by RT-PCR after 8 culture passages (Fig. 1C). Expression of key pluripotency genes was observed both at protein level (transcription factors OCT4, NANOG and SOX2, and surface markers SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81) by immunocytochemistry (Fig. 1D, Table 2) and flow cytometry analysis (Fig. 1E, Table 2), as well as at RNA level (transcription factors *OCT4*, *SOX2*, *REX1*, *NANOG*, *CRIPTO* and *KLF4*) by qRT-PCR (Supplementary Fig. S1A). The iPSC lines also displayed a normal karyotype (46, XX and 46, XY) after more than twenty culture passages (Fig. 1F). The cells also had the capacity to form derivatives of all three germ layers (endoderm, mesoderm and ectoderm) upon embryoid body differentiation (Fig. 1G, Table 2). Mycoplasma testing by a colorimetry assay revealed a negative result (Supplementary Fig. S1B). We confirmed the presence of the two mutations in the iPSC lines by Sanger sequencing (Supplementary Fig. S1C); and we also confirmed by DNA fingerprinting analysis that the lines were derived from the patients´ fibroblasts. In addition, methylation analysis of the promoters of the pluripotency associated genes, *OCT4* and *NANOG*, revealed a heavy methylation in the original fibroblasts and an almost complete demethylation in the iPSC lines (Supplementary Fig. S1D).

Materials and methods

Non-integrative reprogramming of mutant MMAB fibroblasts into iPSC

The present study included available fibroblasts from two MMA *cblB* patients with defects in the *MMAB* gene (Table 1). Experimental protocols were approved by the Institutional Ethical Committee of the Universidad Autónoma de Madrid according to Spanish and European Union legislation, and informed consents were obtained from the legal care-givers. Fibroblasts were reprogrammed using the CytoTune™ iPS Reprogramming kit (Life Technologies) following the manufacturer's instructions. iPSCs were maintained and expanded both on feeder layers and on feeder-free layers as previously described (Alonso-Barroso et al., 2017).

Phosphatase alkaline analysis

Phosphatase alkaline analysis was performed as previously described (Alonso-Barroso et al., 2017).

Detection of Sendai virus genome and transgenes

After 8 passages, iPSC lines were tested for Sendai virus (SeV) residues as described (Alonso-Barroso et al., 2017). PCR was performed using the primers indicated in Table 3 and following the instructions as recommended by the manufacturer. In Fig. 1 panel C: C+: transduced cell pool at passage zero; C−: non-template control.

Immunofluorescence analysis

Immunofluorescence analysis was performed as previously described (Alonso-Barroso et al., 2017). In Fig. 1 scale bars: 100 μm.

Flow cytometry analysis

We analysed the pluripotency-associated marker SSEA-4 by flow cytometry as described (Alonso-Barroso et al., 2017).

Bisulfite sequencing

Bisulfite modification of genomic DNA was performed as previously described (Alonso-Barroso et al., 2017). Converted DNA was amplified by PCR using primers previously published (Freberg et al., 2007) and Immolase™ Red DNA Polymerase (Bioline). In Supplementary Fig. S1D each horizontal row of circles represents the methylation status of each CpG in one clone. Open circles indicate unmethylated CpG dinucleotides and filled circles, methylated.

In vitro differentiation

iPSC colonies were first cultured in suspension so that they form large aggregates called embryoid bodies, and *in vitro* differentiation was performed as described (Alonso-Barroso et al., 2017).

Quantitative PCR analysis

Quantitative PCR analysis was carried out as described (Alonso-Barroso et al., 2017) and the expression levels of several pluripotency associated genes (*OCT4, SOX2, REX1, NANOG, CRIPTO* and *KLF4*) were quantified. Primer sequences were described by Aasen et al., 2008 (Table 3).

Mycoplasma detection

Cells were screened for mycoplasma contamination using a colorimetric assay, PlasmoTest™ Mycoplasma Detection Kit (InvivoGen), following the manufacturer's protocol. In Supplementary Fig. S1B: negative control (C−); positive control (C+).

Mutation analysis

Genomic DNA from the two patients-derived fibroblasts and iPSCs was isolated using MagNA Pure Compact DNA Isolation kit and MagNA Pure Compact instrument (Roche). Subsequently, amplification by PCR of the *MMAB* regions containing the mutations was carried out using the primers indicated in Table 3, and amplified PCR fragments were sequenced in an ABI3730 sequencer (Applied Biosystems).

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
MMAB35-FiPS4F4 (UAMi002-A)	MMAB35-4	Female	Died at 4 years of age	Caucasian	c.287T➔C (p.Ile96Thr) and c.584G➔A (p.Ser174Cysfs*23)	MMA *cblB* type
MMAB44-FiPS4F11 (UAMi003-A)	MMAB44-11	Male	11 years asymptomatic	Caucasian	c.287T➔C (p.Ile96Thr) and c.584G➔A (p.Ser174Cysfs*23)	MMA *cblB* type

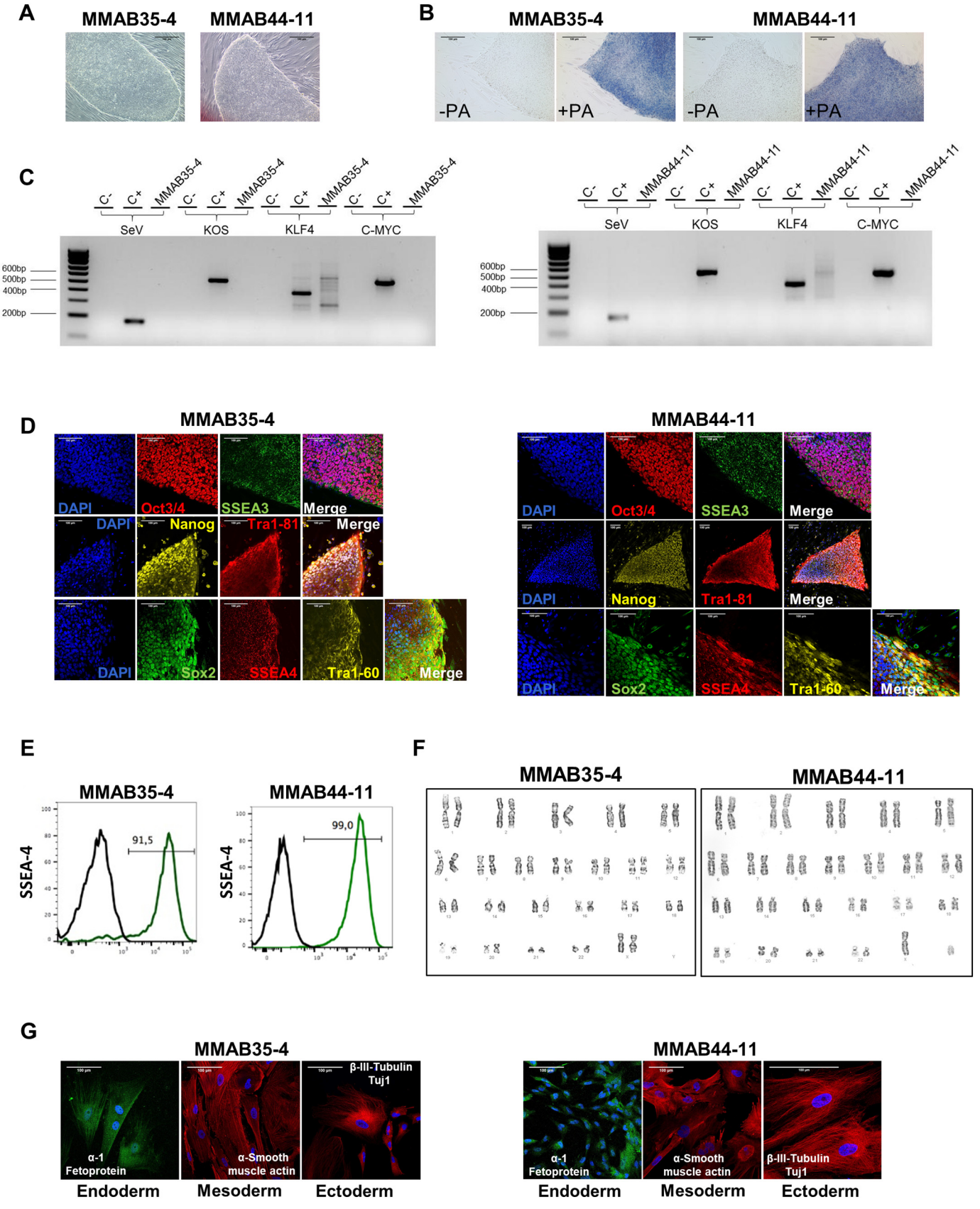

Fig. 1. Generation and molecular and functional characterization of the iPSC lines.

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	Visual record of the lines: normal	Fig. 1 panel A
Phenotype	Qualitative analysis	Assess staining/expression of pluripotency and cell markers: OCT4, NANOG, SOX2, SSEA-3, TRA-1-81, SSEA-4 and TRA-1-60	Fig. 1 panel D
	Quantitative analysis	Assess % of positive cells for SSEA-4. MMAB35-4: 91.5%. MMAB44-11: 99%	Fig. 1 panel E
Genotype	Karyotype (G-banding) and resolution	46XX and 46XY Resolution 450–500	Fig. 1 panel F
Identity	STR analysis	16 sites tested and all of them matched	Submitted in archive with journal
Mutation analysis (if applicable)	Sequencing	c.287T➔C (p.Ile96Thr) and c.584G➔A (p.Ser174Cysfs*23)	Supplementary Fig. S1 panel C
	Southern Blot OR WGS	Not performed	
Microbiology and virology	Mycoplasma	Mycoplasma testing by a colorimetric assay: negative	Supplementary Fig. S1 panel B
Differentiation potential	Embryoid body formation	Expression of smooth muscle actin, β-III-tubulin Tuj1 and α-1 fetoprotein	Fig. 1 panel G
Donor screening (optional)	HIV 1 + 2 Hepatitis B, Hepatitis C	Not performed	No
Genotype additional info (optional)	Blood group genotyping	Not performed	No
	HLA tissue typing	Not performed	No

Karyotype analysis

Karyotype analysis of the iPSC lines was carried out using cells with more than twenty culture passages which were processed using standard cytogenetic techniques as described (Alonso-Barroso et al., 2017).

DNA fingerprinting analysis

DNA fingerprinting analysis was performed as previously described (Alonso-Barroso et al., 2017).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2018.03.021.

Acknowledgments

This work was funded by the *Instituto de Salud Carlos III (Ministerio de Economía y Competitividad* (MINECO) co-funded with the European Regional Development Fund, grant PI13/01239) and the *Fundación Isabel Gemio*. The authors wholeheartedly thank the Spanish families affected by MMA who actively participated in this work and Mª Luisa de la Torre (Instituto de Genética Médica y Molecular del Hospital Universitario de La Paz, Madrid, Spain) for excellent assistance with the karyotype analyses. The *Centro de Biología Molecular Severo Ochoa* receives an institutional grant from the *Fundación Ramón Areces*.

Table 3
Reagents details.

Antibodies used for immunocytochemistry

	Antibody	Dilution	Company Cat # and RRID
Pluripotency markers	Mouse IgG anti-OCT4	1:60	Santa Cruz Cat# sc-5279, AB_628051
	Rat IgM anti-SSEA-3	1:3	Hybridoma Bank Cat# MC-631, AB_528476
	Rabbit IgG anti-SOX2	1:100	Fisher Thermo Scientific Cat# PA1-16968, AB_2195781
	Mouse IgG anti-SSEA-4	1:3	Hybridoma Bank Cat# MC-813-70, AB_528477
	Mouse IgM anti-TRA-1-60	1:200	Millipore Cat# MAB4360, AB_2119183
	Goat IgG anti-NANOG human	1:25	R&D Cat# AF1997, AB_355097
	Mouse IgM anti-TRA-1-81	1:200	Millipore Cat# MAB4381, AB_177638
Differentiation markers	Rabbit IgG anti-α-Fetoprotein	1:400	Dako Cat# A0008, AB_2650473
	Mouse IgG anti-β-III-Tubulin Tuj1	1:500	Covance Cat# MMS-435P, AB_231377
	Mouse IgG anti-α-smooth muscle actin	1:400	Sigma-Aldrich Cat# A5228, AB_262054
Secondary antibodies	Alexa 555 Donkey anti-Mouse IgG	1:200	Thermo Fischer Cat# A-31570, AB_2536180
	Alexa 488 Goat anti-Rat IgM	1:200	Thermo Fischer Cat# A-21212, AB_2535798
	Alexa 488 Donkey anti-Rabbit IgG	1:200	Thermo Fischer Cat# A-31572, AB_162543
	Alexa 555 Donkey anti-Mouse IgG	1:200	Thermo Fischer Cat# A-31570, AB_2536180
	Alexa 647 Goat anti-Mouse IgM	1:200	Thermo Fischer Cat# A-21238, AB_2535807
	Alexa 647 Donkey anti-Goat IgG	1:200	Thermo Fischer Cat# A-21447, AB_2535864
	Alexa 55 Donkey anti-Mouse IgM Cy3	1:200	Jackson Cat# 715-165-140,

Primers

	Target	Forward/reverse primer (5′-3′)
Reverse transcription-PCR	*SeV* genome	GGATCACTAGGTGATATCGAGC/ACCAGACAAGAGTTTAAGAGATATGTATC
	KOS transgene	ATGCACCGCTACGACGTGAGCGC/ACCTTGACAATCCTGATGTGG
	KLF4 transgene	TTCCTGCATGCCAGAGGAGCCC/AATGTATCGAAGGTGCTCAA
	c-MYC transgene	TAACTGACTAGCAGGCTTGTCG/TCCACATACAGTCCTGGATGATGATG
Pluripotency markers (qPCR)	*OCT4*	GGAGGAAGCTGACAACAATGAAA/GGCCTGCACGAGGGTTT
	SOX2	TGCGAGCGCTGCACAT/TCATGAGCGTCTTGGTTTTCC
	NANOG	ACAACTGGCCGAAGAATAGCA/GGTTCCCAGTCGGGTTCAC
	CRIPTO	CGGAACTGTGAGCACGATGT/GGGCAGCCAGGTGTCATG
	REX1	CCTGCAGGCGGAAATAGAAC/GCACACATAGCCATCACATAAGG
	KLF4	CGAACCCACACAGGTGAGAA/GAGCGGGCGAATTTCCAT
House-keeping genes (qPCR)	*GAPDH*	GCACCGTCAAGGCTGAGAAC/AGGGATCTCGCTCCTGGAA
Targeted mutation analysis/sequencing (PCR)	*MMAB exon 3 MMAB exons 6-7*	GCCTGGATGACAGAGTGACTGTT/ATAAGGGCTGACAACCTCCGAGG CGCCATGGTCTGGTGGGTGAT/TCAGAG ATGGCCCTGCTGTA

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